# VIRGINIA'S 2004 MOSQUITO TESTING PROTOCOL

Increases in the cost of testing without further increases in the size of the WNV testing budget has required Virginia to maximize its mosquito testing efficiency and minimize unproductive testing. This increased efficiency/productivity will prevent the laboratories from overspending their mosquito testing budgets and will also permit the laboratories to devote some of their resources towards testing certain mosquito species from LaCross Encephalitis (LAC) endemic regions for LAC. Increased testing efficiency will be accomplished by only targeting appropriate vector species for testing at appropriate times of the season, and by reducing the number of pools tested by laboratory enforcement of the minimum **pool size** to **not less than 25 mosquitoes** for most **bridge vector** species. Whenever possible, surveillance programs should pool mosquitoes to maximize pool size and minimize the number of pools submitted.

The new mosquito testing protocol will focus on testing **primary vector** species as a means of estimating the **minimum** (mosquito) **infection rates** (**MIR**)* for WNV and EEE. The local **MIR** provides a good estimate of the potential for WNV or EEE to cause human disease in that locality, and the **MIR** can be compared from location-to-location and from year-to-year.

**Primary vector** species are always the main factor driving both WNV and EEE activity. **Bridge vector** species generally do not become infected with these viruses before the infection rate has reached high levels in populations of the **primary vector** species. Therefore, early season testing of low-potential **bridge vector** species (e.g., ***Oc. canadensis***, other floodwater ***Ochlerotatus*** species, and ***Anopheles*** species) is unlikely to produce positive results and should not be done.

Several seasons of mosquito testing in Virginia and other Mid-Atlantic states indicates that ***Culex pipiens*** and ***Cx. restuans*** are the most important **primary vectors** of WNV, and that the container breeding ***Aedes*** and ***Ochlerotatus*** species are the next most likely to be found carrying WNV. All other potential **bridge vector** species are not as likely to be found infected with WNV until August, and these other species should not be tested prior to the beginning of August, or before WNV has been detected at significant levels in local **primary vector** populations. For example, most potential **bridge vector** species will not become infected with WNV until the local **primary vector** population reaches a **MIR** of **10**. The **MIR trigger thresholds**** will be set for WNV and EEE at 5 per thousand and 1 per thousand, respectively.

***Minimum Infection Rates (MIR)** should be calculated at the local level by mosquito surveillance personnel. The calculation should be made as a **weekly, county** or **city MIR,** i.e., the number of infected pools of the **primary vector** species (e.g., *Culex pipiens*) per 1000 mosquitoes tested in a jurisdiction during any given week (week = period from Sunday through the following Saturday). The **MIR** is calculated as the number of pools testing positive for either WNV or EEE, divided by the total number of that species submitted for testing during the week, multiplied by 1000.

$$\text{MIR} = \left( \frac{\text{Number of Positive Pools}}{\text{Number of Mosquitoes Tested}} \right) \times 1000$$

****Trigger Threshold MIR –** A predetermined level of weekly, county or city MIR at which time a change in surveillance practices may be made.

**WNV Testing;** testing to be conducted on mosquitoes collected statewide**:**

**Primary vector** and **bridge vector** species that can be tested in **pools of 10 to 50 mosquitoes** during the entire testing season (when possible, make larger pools).

***Culex pipiens***
***Culex restuans***
***Aedes albopictus***
***Ochlerotatus triseriatus***
***Ochlerotatus japonicus***

**Primary vector/bridge vector** species that should be tested in a **minimum pool size of 25** mosquitoes until August 1st or until the local **trigger threshold MIR*** in ***Culex pipiens*** or ***Cx. restuans*** reaches **5,** whichever comes first, after which the minimum pool size can be as little as **10 mosquitoes** if necessary.

***Culex salinarius***
***Culex erraticus***

Potential **primary vector/bridge vector** species that may be tested between **June 15th** and **September 15th**. **Minimum pool size is 25 mosquitoes**.

***Culiseta melanura***

Species that will not be tested until after August 1st or until the local **trigger threshold MIR*** in ***Culex pipiens*** or ***Cx. restuans*** reaches **5,** whichever comes first. **Minimum pool size is 25 mosquitoes**.

***Aedes vexans***
***Ochlerotatus trivittatus***
***Ochlerotatus sollicitans***
***Ochlerotatus taeniorhynchus***
***Ochlerotatus sticticus***
***Ochlerotatus canadensis***
***Ochlerotatus atlanticus/tormentor***
***Ochlerotatus infirmatus***
***Anopheles punctipennis***
***Anopheles quadrimaculatus***
***Coquillettidia perturbans***

***Trigger Threshold MIR for WNV** - The trigger threshold for WNV will be **5** WNV positive pools of *Cx. pipiens* or *Cx restuans* per 1000 tested within a jurisdiction (county or city) during a given week.

**EEE Testing;** limited to mosquitoes from jurisdictions of Virginia's coastal plane [i.e., areas east of I-95] unless activity is detected elsewhere in the state**:**

**Primary vector/potential bridge vector** species that can be tested in **pools of 25 to 50 mosquitoes** during the **entire testing season**.

***Culiseta melanura***

**Primary vector/bridge vector** species that should be tested in a **minimum pool size of 25** mosquitoes until August 1st or until the local **trigger threshold – MIR*** in ***Cs. melanura*** reaches **1,** whichever comes first, after which the minimum pool size can be as little as **10 mosquitoes** if necessary.

***Culex salinarius***
***Coquillettidia perturbans***

**Bridge vector** species that can be tested in **pools of 10 to 50 mosquitoes** during the **entire testing season**.

***Aedes albopictus***

**Bridge vector** species that will not be tested until after August 1s,t or until the local **trigger threshold MIR*** in ***Culiseta melanura*** reaches **1,** whichever comes first. **Minimum pool size is 25**.

***Aedes vexans***
***Ochlerotatus sollicitans***
***Ochlerotatus taeniorhynchus***
***Ochlerotatus canadensis***
***Ochlerotatus atlanticus/tormentor***
***Ochlerotatus infirmatus***
***Anopheles crucians***
***Anopheles quadrimaculatus***
***Anopheles punctipennis***
***Culex erraticus***

***Trigger Threshold MIR for EEE** - The trigger threshold will be **1** EEE positive pool of *Cs. melanura* per 1000 tested within a jurisdiction (county or city) during a given week. Once the trigger threshold has been reached by a single jurisdiction within the Tidewater Area, all other Tidewater jurisdictions may alter their mosquito testing procedures.

**Testing for Other Mosquito Borne Viruses:**

**LaCross Encephalitis (LAC) Testing**; limited to mosquitoes from Virginia's mountain jurisdictions [west of State Route – 29] unless activity is detected elsewhere in the state**:**

**Vector** species that can be tested for LAC in **pools of 10 to 50 mosquitoes** during the entire testing season.

***Aedes albopictus***
***Ochlerotatus triseriatus***
***Ochlerotatus japonicus***

**Vector** species that can be tested for LAC in **pools of 25 to 50 mosquitoes** after LAC has been detected in a jurisdiction.

***Ochlerotatus canadensis***

**St. Louis Encephalitis (SLE) Testing;** limited mosquitoes from local geographic areas [counties or cities] surrounding where SLE infections have been identified in human patients**:**

**Primary vector** and **bridge vector** species that can be tested in **pools of 10 to 50 mosquitoes**.

***Culex pipiens***
***Culex restuans***
***Aedes albopictus***
***Ochlerotatus triseriatus***
***Ochlerotatus japonicus***

**Primary vector** and **bridge vector** species that can be tested in **pools of 25 to 50 mosquitoes**.

***Culex salinarius***
***Culex erraticus***

The above listed protocol for arboviral testing may be altered in the event of special or mitigating circumstances. Any surveillance program wishing to test species or pool sizes outside the protocol specifications should contact and consult with Dr. David Gaines at VDH (tel. 804- 864-8141, david,gaines@vdh.virginia.gov), as well as contact and communicate with personnel in charge of the testing laboratory you send your specimens to, prior to doing so.

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